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Baseline

Incidence of microplastics in Indian anchovy *Stolephorus indicus* from Tuticorin, Southeast coast of India

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A R T I C L E I N F O

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A B S T R A C T

In the present study, the occurrence of microplastics (MPs) in the gut, gill, and muscle of edible fish *Stolephorus indicus* sampled from Tuticorin coastal regions of Tamilnadu, India was investigated. We recorded a total of 689 MPs which includes 510 and 179 MPs from males and females respectively. The total abundance of MPs was significantly ($P < 0.05$) higher in the gut followed by gills and muscle. The sex-wise distribution of average MPs showed high in the females' gut and compared to that in males. Further, the length wise distribution of MPs was higher in the muscle in both male and female fish, followed by other organs. The predominance of MPs in tissues were transparent and blue colour with fibers and fragments in both males and females. Besides, polyethylene terephthalate and nylon were evidenced by the Fourier-transform infrared spectroscopy spectrum in all organs of fishes.

The marine ecosystem plays a pivotal role in the world for regulating climate, production of oxygen, habitat for various living beings, and providing nutritional security for nations. In recent days marine environments have been contaminated by various pollutants like heavy metals, pesticides, hydrocarbons, microplastics (MPs), etc. (Kinigopoulou et al., 2022; Zhang et al., 2022). Among various contaminants in the marine environment, nanoplastics (<100 nm) and MPs (>100 mm to <5 mm) are abundant pollutants that affect marine organisms (Oriekhova and Stoll, 2018; Wagner and Reemtsma, 2019; Du and Wang, 2021). MPs from industrial and domestic products are also significant plastic inputs, as they are released into sewage systems and eventually run into the oceans (McDevitt et al., 2017). MPs are being originated directly as primary MPs from industries and indirectly by the larger plastic particles fragmented via environmental factors like microbes, UV-radiation from sunlight, and physical abrasion processes (Sun et al., 2020). MPs are a great concern in aquatic environments due to their buoyancy, slow degradability, adherence to organic and inorganic contaminants, and colonization of pathogenic microbes (Elgarahy et al., 2021; Sooriyakumar et al., 2022).

Fate of MPs has been reported to be harmful to a wide range of marine organisms in terms of trapping in gills, scales, false feeding, intestinal damage, poor digestion, growth, survival, etc. (Alak et al., 2022; Ahmed et al., 2023). Ingestion of MPs by marine organisms like mussels (Patterson et al., 2019; Kazour and Amara, 2020), decapod crustaceans (Valencia-Castañeda et al., 2022; Paez-Osuna et al., 2023), and fish has been documented (Alomar et al., 2017; Kumar et al., 2018). These ingested MPs can cause different adverse effects on feeding behavior, reduction of predatory performance, reproduction, and energy budget, inflammatory responses, histological changes, DNA damage, cytotoxicity, physical damage, and mortality in aquatic organisms (Hu and Palic, 2020; Du et al., 2020; Chang et al., 2022). Moreover, the occurrence of MPs in seafood has also been documented, which can transfer to human consumers as a biomagnification process (Mercogliano et al., 2020). Besides, there is no specific regulation or legislation related to contamination of MPs in foods including seafood, however, continuous exposure to MPs and its associated chemicals can produce potential health risks like oxidative stress, metabolic disorder, cancer, developmental toxicity neurotoxicity, etc., to human consumers (Campanale

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et al., 2020; Gamarro and Costanzo, 2022; Li et al., 2023). Earlier studies have shown the occurrence of MPs in edible fishes such as *Rastrilleger kanagurta, Lateolabrax maculatus,* and *Cyprinus carpi*. (Kumar et al., 2018; Su et al., 2019; Barboza et al., 2020) and its possible transfer to human consumers (Kannan and Vimalkumar, 2021; Huang et al., 2021). Indian anchovy, *S. indicus* constitutes the most important pelagic resource which is distributed in tropical and subtropical regions of the Indo-Pacific. This species serves as an important food fish in India, which contributes 9.2 % of Indian marine fisheries (CMFRI, 2016). The main food for anchovy is plankton and it serves as a link between plankton and other tropic animals (Alizada et al., 2020).

Tuticorin District of Tamil Nadu, India, borders the southern part of the Gulf of Mannar. This city is one of India's major fishing ports on the southeast coast. Tuticorin City has great interest in the investigation of pollutants due to consisting of many major industries, involved in the production of chemicals, petrochemicals, and plastics. The city generates wastewater of nearly 18 million liters per day which includes domestic sewage and industrial effluents (Malarvannan and Balamurugan, 2018). MPs contamination in the seawater and sediments is primarily linked to sewage release and various commercial activities such as fishing, development of the coastline, effluent from paper mills, agriculture activities and litter by tourism activities get transported to the beaches and cause plastic pollution (Katsumi et al., 2020; Jesuraja et al., 2021; Khadanga et al., 2022). In this district 1, 81, 200 hector agriculture activities associated with the utilization of 22,649 metric tons of fertilizers, 58 metric tons of dust, and 7920 l of liquid pesticides per year (DAC, 2021; TDC, 2024), and the arrival of 5.73 million tourist visitors (MTGI, 2014) which are the possible factors for MPs pollution. Earlier studies revealed the occurrence of MPs in the water, sediments, and biota sampled from the coastal environment of Tuticorin (Kumar et al., 2018; Patterson et al., 2019; Sathish et al., 2020; Sathish and Patterson, 2023). However, the occurrence of MPs in the edible fish Indian anchovy *S. indicus* has not been studied from Tuticorin coastal regions. Hence, the present study aimed to evaluate the gender-based occurrence and distribution of MPs in the different organs and tissues (gills, gut, and muscle) of *S. indicus* sampled from the Tuticorin coastal region.

In the present study, a total of 25 individual fish *S. indicus* were sampled from the Threspuram fish landing center (80° 48′ 456″ N - 78° 09′ 485″ E) of Tuticorin (Fig. S1a supplementary material). The collected fish were kept in a sterile sample canister and transported to the laboratory for further examination. The sex and morphometry of sampled fish were measured and recorded as six females (mean length 12.6 cm and weight 15.0 g) and 19 males (mean length 12.8 cm and weight 16.5 g) (Fig. S1b & c supplementary material). To keep down contamination and remove the debris, the fish were washed using double distilled water. Once this process was completed, the fishes were photographed using a Canon E300-6D camera for morphological characterization, and the taxonomical identification of fish was performed using the FishBase website (FishBase, 2023) and standard manual (Strauss and Bond, 1990).

The gut, gills, and muscles of fish from each gender were dissected in an aseptic condition, weighed, and transferred to a sterile glass container for further processing. Each organ was digested in 10 % KOH solution at the ratio of 1:10 (weight: volume) for 96 h at 60 °C (Karami et al., 2017), the lipid layer was removed using equal volume of 99.9 % ethanol (Dawson et al., 2020) under ultra-probe sonication, centrifuged at 8000 rpm for 10 min and the supernatant was filtered in a gridded cellulose nitrate membrane filter paper (pore size 0.22 μm) using vacuum filtration assembly. Further, the pellets were dissolved in a high-density solution of potassium chloride followed by centrifuging at 8000 rpm for 10 min to separate the plastic particles in the supernatant and filtered in filter paper and the filtrate was stored separately in a pre-cleaned petri dish for further inspection. MPs in each filter paper were confirmed by hot needle test as prescribed earlier (Lusher et al., 2017), followed by being viewed and photographed under a stereo zoom microscope equipped with a camera (Olympus SZ61TR) at 40× magnification. The morphometric properties (shape, size, and colour) of MPs were examined as per the earlier method (Hidalgo-Ruz et al., 2012). The MPs >100 μm size from each organ of male (gut: 22, gills: 19, and muscle: 10) and female (gut: 10, gills: 5, and muscle: 4) fish were randomly selected for polymer type identification in Fourier-transform infrared (FT-IR) spectroscopy (JASCO FTIR 4700, Japan) equipped with attenuated total reflectance mode (ATR) with a frequency range of 4000 cm^{-1} to 650 cm^{-1} and coding 32 scans with 10 Hz, at a resolution of 4 cm^{-1}. All the acquired wave numbers of spectra were compared and confirmed based on the previous reports (Veerasingam et al., 2021; Smith, 2021; Liu et al., 2022).

Quality control measures were taken to avoid airborne MPs during the sample collection and laboratory extraction and characterization of MPs. In brief, the sampled fish were immediately washed using reverse osmosis water and covered with cotton cloth. The lab manpower wore a 100 % cotton lab coat and nitrile gloves to prevent contamination. All dissection tools, glassware, and containers were rinsed using ultrapure water before use. The microscope, vacuum filtration unit, and ultra-probe sonicator were cleaned using clean cotton cloth before and after use. The blank solution without any fish samples was prepared with the same procedure used for the extraction of MPs from fish samples to ensure MPs contamination free solutions throughout the analysis. Moreover, procedures were performed on the clean workbench placed in a separate contamination free room. The data obtained from all the parameters were expressed as mean ± SD. Significance variation in the abundance and size of MPs in the different organs of male and female fish were determined by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS (20.0) software.

In the present study, the result of the abundance of MPs indicates that the total number of MPs isolated from three different organs (gut, gill, and muscle) was 689 of which 510 and 179 MPs were extracted from male and female fish respectively. The overall average number of microplastic particles of the 25 individuals was 27.6 of all three organs. Further, overall abundance was significantly ($P < 0.05$) high in the gut followed by gills and muscle. The gender-wise distribution of MPs showed high in the female gut compared to the male gut and muscle. Moreover, female gills showed low MPs compared to male gills (Table 1). The overall lengthwise distribution of MPs showed a length range between 2.3 and 153.6 μm and the total length range was recorded in gut 9.6 to 133.1 μm, gills 4.4 to 76.3 μm, and muscle 5.2 to 74.0 μm. MPs extracted from the different organs of males showed the maximum particle size in the gut (153.6 μm), followed by gills (86.0 μm) and muscle (69.4 μm). In the case of females, the maximum length of the MPs was noticed in muscle (88.7 μm) followed by gut (68.0 μm), and gills (45.6 μm). In males, the mean length of the particle in muscle (53.0 μm) was significantly ($P < 0.05$) higher compared to the gut (38.1 μm), and gills (27.7 μm). While the mean length was significantly ($P < 0.05$) high in the muscle of females (42.0 μm) followed by the gut (26.6 μm), and gills (22.7 μm) of females. While, the overall mean length was found to be higher in the gut, gills, and muscles of males compared to MPs in female fish (Table 1). These results indicated that the female intestinal tract had more MPs due to their more feed consumption for their high energy requirement during the spawning seasons in the MPs contaminant environments (Foltz and Norden, 1977; Lambert and Dutil, 2000). Some fishes select MPs as their food because of the size and colour of the particles that resemble their prey like phytoplankton and zooplankton (Ory et al., 2017; Li et al., 2021). Likewise, Horton et al. (2018) and Senturk et al. (2023) reported higher MPs ingestion by female fishes (*Rutilus rutilus* and *Syngnathus acus*) compared to males. These conditions can cause a false feeling of satiation (Rummel et al., 2016; Tanaka and Takada, 2016) which could be the main reason for MPs in the gut and it may cause starvation and physical damage. Besides, the MPs can be observed by the intestinal epithelial cells, followed by transfer to blood vessels and internal muscular tissues. The skin lesions alterations can possible entry of MPs into muscle (Jabeen et al., 2018; Barboza et al., 2020). Moreover, gills are being exposed directly to the

Table 1
Abundance and lengthwise of MPs in the different organs of *S. indicus*.

Abundance	Gender	Total No. of MPs			Total	Average No. of MPs/individual		
		Gut	Gills	Muscle		Gut	Gills	Muscles
	Male	223	190	97	510	11.7 ± 12.2 ^{a}	10.0 ± 7.5^{ab}	5.1 ± 8.0^{b}
	Female	98	49	32	179	16.3 ± 9.0^{a}	8.2 ± 7.8^{ab}	5.3 ± 4.7^{b}
	Overall	321	239	129	689	12.8 ± 11.5^{a}	9.6 ± 7.4^{ab}	5.2 ± 7.2^{b}
	Overall							27.60 ± 16.21

Lengthwise distribution	Gender	Length of MPs (μm)						Mean length (μm)		
		Gut		Gills		Muscle		Gut	Gills	Muscle
		Min.	Max.	Min.	Max.	Min.	Max.			
	Male	11.5	153.6	3.9	86.0	5.9	69.4	38.1 ± 54.8^{b}	27.7 ± 38.9^{b}	53.0 ± 47.2^{a}
	Female	2.3	68.0	6.1	45.6	3.4	88.7	26.6 ± 25.9^{b}	22.7 ± 26.9^{b}	42.0 ± 48.2^{a}
	Overall	9.6	133.1	4.4	76.3	5.2	74.0	34.6 ± 48.0^{b}	26.5 ± 36.3^{b}	50.3 ± 47.4^{a}
	Overall									34.9 ± 45.0

Mean ± SD; ($n = 19$ and $n = 6$ for males and females fish respectively). Mean values within the same column sharing the same superscript are not significantly different ($P > 0.05$).

environmental matrices while doing respiration thus the MPs can passively enter their gill chambers and they will adhere to them (Porcino et al., 2022). Exposure of MPs can produce oxidative stress, tissue damage, behavioral changes, poor digestion, growth retardation, genotoxicity, changes in immune gene expression, and reproduction in aquatic organisms (Bhuyan, 2022). In the present study, the occurrence of MPs with higher length in the gut indicates that the higher length of MPs ingested by the fishes trapped in the gut and small particles reached the muscle. Furthermore, the incidence of MPs with higher mean length in the muscle tissue compared to other organs indicates that the MPs reach the muscle up to a certain maximal length. Earlier studies observed that the MPs particle size ranged between 50 and 5000 μm, 50 to 5000 μm, and 50–1000 μm frequencies in the gut, gills, and muscle of various marine fishes (*Scomberomorus guttatus*, *Mullus barbatus*, *Alosa immaculate*, *Sardina pilchardus*, *Engraulis encrasicolus*, *Trachurus trachurus*, *Osteogeneiosus militaris*, *Ethmalosa fimbriata*, *Pseudotolithus senegalensis*, and *Galeoides decadactylus*) respectively (Atamanalp et al., 2021; Hossain et al., 2023; Lopes et al., 2023; Pradit et al., 2023; Amponsah et al., 2024). Furthermore, earlier studies on the occurrence of MPs per individuals in the gut and gills of fishes such as *Gobionellus occidentalis*, *Elops lacerta*, *Mugil bananesis*, *Chrysichthys nigrodigitalus*, *Cynoglossus senegalensis*, *Sarotherodon melanotheron*, *Galeoides decadactylus*, *Pseudotolithus senegalensis*, *Ethmalosa fimbriata*, and *Apsilus fuscus* (Amponsah et al., 2024) showed the lower numbers with higher length compared to the present study. Daniel et al. (2020) and Atamanalp et al. (2021) reported a lower number of MPs per individuals with higher particles

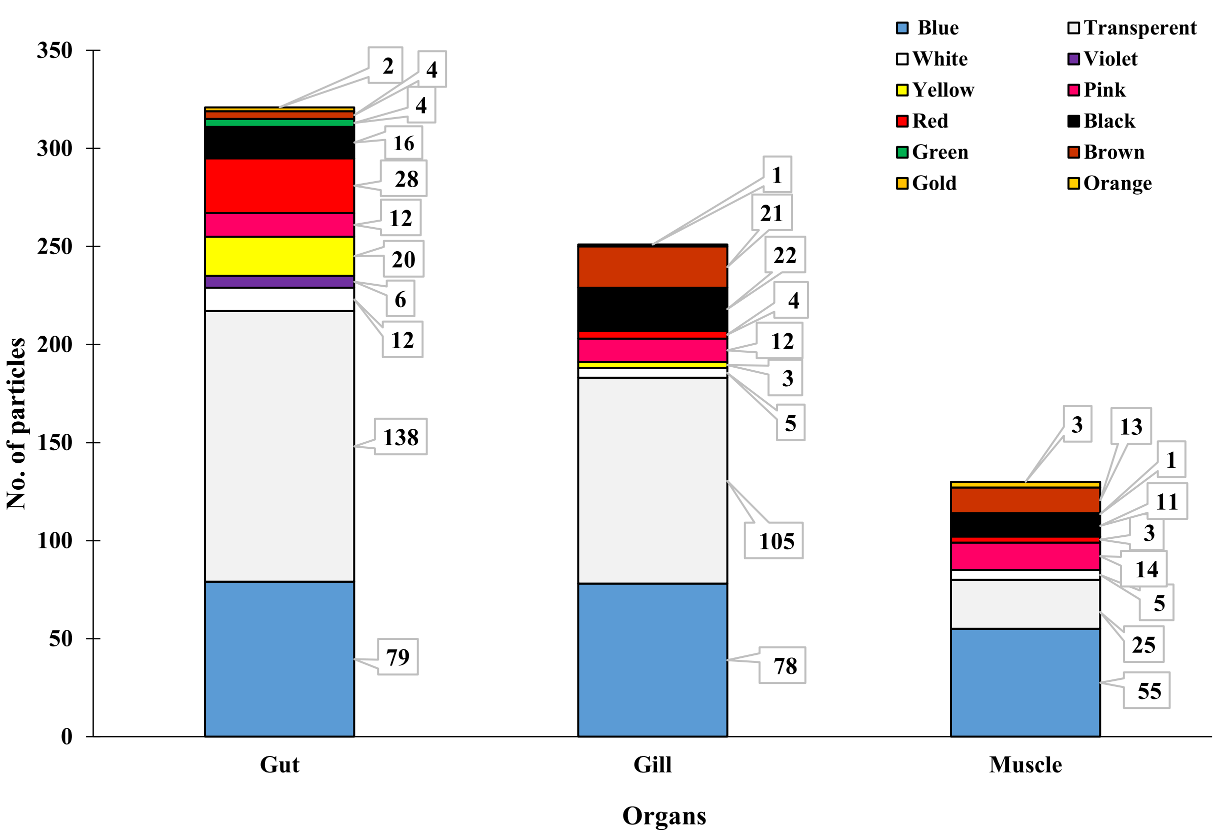

Fig. 1. Colour wise distribution of MPs in both male and female fish (*S. indicus*).

length range in the gill, viscera, muscle, and skin of *R. kanagurta*, *Megalaspis cordyla*, *Sardinella longiceps*, *Sardinella gibbosa*, *S. indicus*, *Dussumieria acuta*, *Thryssa dussumieri*, *Sphyraena, obtusata*, *Anodontostoma chacunda, Mullus barbatus*, and *Alosa immaculata* compared to the current study. Nonetheless, a higher number of MPs in the edible tissues of *Parexocoetus mento* compared to the present study was recorded earlier (Abidin et al., 2021).

In the current study, Fig. 1 shows the different colour of MPs recorded in different organs of the 25 fish individuals. Overall, the transparent particles were higher (268), followed by blue (212), black (49), pink (38) and brown (38). In this context, the dominant particle was noticed as transparent (194) followed by blue (163), black (39), brown (31), and red (27) in males. While, the transparent (74) particles were dominant in females, followed by blue (49), pink (12), yellow (11), and white (11). Amidst MPs isolated from both sexes, the maximum observed colour was transparent and blue. Further, the transparent colour was high in the gut (45 %) of males, followed by gills (40 %) and blue colour was highly observed in muscle (44 %). In females, transparent MPs were high in gills (48 %), followed by gut (40 %) and muscle (37 %). Besides, the blue colour was the second dominant MPs in all organs of *S. indicus* compared to other colored MPs. This result indicated that some phytoplankton and zooplankton are transparent or white, hence, the fish might be recognized these transparent and white colour MPs as food sources that lead to false feeding. Likewise, the dominant level of transparent and white MPs was recorded earlier in nine commercially important pelagic marine fishes including *S. indicus, R. kanagurta, M. cordyla, S. longiceps, S. gibbosa, D. acuta, T. dussumieri, S. obtusata,* and *A. chacunda* (Daniel et al., 2020), which are highly edible for human consumers. Koongolla et al. (2020) found a greater amount of colorless and white MPs in the gastrointestinal tract of 12 fish species. Besides the higher selectivity of the blue colour and the movement by the ocean, the current and shape of the MPs are similar to their prey and it misleads maximum of the marine organism (Fernandez-Ojeda et al., 2021; Scacco et al., 2022). Previous studies in fishes (*Oreochromis mossambicus, Terapon jarbua, Acanthurus dussumieri,* and *Mugil* sp.,) have reported that blue colour dominance in gut, gills, and muscles (Guven et al., 2017; Kazour and Amara, 2020; Naidoo et al., 2020; Ghosh et al., 2021). Many of the studies reported that mostly dark-colored MPs as like the food consumed by the fishes (worms and algae) (Bessa et al., 2018; Nelms et al., 2018). The specific colour preference of microplastics by the fish has been accredited to the eating habits of the fish groups (Pappoe et al., 2022). Hence, colour has been considered an essential parameter affecting the feeding habit and leads uptake of MPs by marine organisms (Abayomi et al., 2017).

In the present study, five different shapes of MPs (fiber, fragment, pellet, foam, and film) were recorded in the different organs of the 25 individual fishes. Overall, the fiber shape is the most dominant (481) in the fish *S. indicus*, followed by fragment (99), pellet (82), foam (19), and film (8). From this, a high number of all five shapes was recorded in the gut followed by gill and muscle. In the case of males, the dominant shape was fiber (368), followed by fragment (70), pellet (57), foam (10), and film (5). As in females, the highest shape was fiber (113), followed by fragment (29), pellet (25), foam (9), and film (3) (Fig.S2a &b; Fig.S3a supplementary material), which agrees with the previous study, fiber shaped MPs abundance was the most found dominant in digestive tracts of fishes (Zhang et al., 2020; Wu et al., 2020). This result suggests that the main sources of microfibers to the fish might be the fragments of fishing gears (Andrady, 2011) and domestic cloth washing discharge (Cesa et al., 2017; Li et al., 2022; Pradit et al., 2023). In the present study, fragments are the most found shape in all tested organs of *S. indicus* after the fiber this suggests that the fragments originated from macro plastics in the environment (Massos and Turner, 2017). Similarly, Sun et al. (2020) noticed that the fragment was the most noticed shape of MPs in the digestive tracts of fish *Oreochromis niloticus*. Also, a high level of fragments was recorded in the gut of fishes *Decapterus maruadsi*, *Pampus argenteus*, *Collichthys lucidus*, and *Setipinna tenuifilis* (Wu et al., 2020). Furthermore, the overall occurrence of MPs was in the form of fiber and fragments in all studied organs and tissues (gut, gills, and muscle) studied fishes that denote fish might be exposed and consumed more levels of these MPs as false feeding due to their shapes resemble with phytoplankton and zooplankton, hence, these two types of MPs have a high relationship with all studied organs. Likewise, the dominant level of fibers and fragments in the gut, gills, and muscle of marine fishes such as *Osteogeneiosus militaris*, *E. lacerta*, *C. nigrodigitalus*, *S. melanotheron*, and *G. decadactylus* reported earlier (Pradit et al., 2023; Amponsah et al., 2024).

The polymer nature identification of MPs can indicate their source of origin. In the present investigation, the ATR-FT-IR spectrum showed the presence of polystyrene (PS), nylon, polycarbonate (PC), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) in the male fish gut. Whereas, the gut of female fish showed nylon, PET, and PVC. In this context, PET and nylons were the most commonly observed polymers in the gut, gills, and muscles of both male and female fishes (Table S1; Fig. S3b supplementary material), which indicates that these polymers might have fragmented from macro plastics sources like fishing gears, textiles, straws, films, personal care products, single use plastic cups, electronics waste, and other domestic wastes (Coyle et al., 2020) and get accumulated in the fish by exposure and ingestion. Likewise, the presence of nylon, PS, and PET in the liver, gills, and digestive tract of fishes such as *Taurulus baublis, Eleutheronema tetradactylum, Trichiurus lepturus* have been recorded earlier (Piskuła and Astel, 2023; Hidayati et al., 2023). Also, the dominance of PET and nylon in the viscera and gills of fishes *E. lacerta* and *Dicentrarchus labrax* have been reported (Reinold et al., 2021; Amponsah et al., 2024).

There is limited research on the gender based comparison of MPs occurrence in fishes. The present investigation revealed a high occurrence of MPs in the gut followed by gills and muscles of *S. indicus* in both genders. However, female fish guts had a higher level of MPs than males, which suggests that female fish might consumed more MPs as false feed. Also, the muscles of both male and female fish were recorded with MPs with maximum mean length, which indicates the vulnerability of this edible fish species to MPs contamination that led to seafood safety. Transparent and blue in colored MPs with fiber and fragment shapes were dominant in both males and females, which might have fragmented from macro plastics like fishing gears, textiles, domestic waste, and other single use plastics with evidence of nylon, PS, PET, and PVC nature in spectral analysis. As limitations in this study, the seasonal based investigation is needed to understand the high incidence of MPs in the males and females of *S. indicus.* Besides, fish was sampled from the fish landing center, hence, there might be possibilities of MPs contamination in the surface of fish at the time of harvesting to market. Therefore, sampled fish must be washed using pre-filtered double distilled or reverse osmosis water immediately after sampling to reduce the MPs contamination as better quality control.

CRediT authorship contribution statement

Velusamy Gayathri: Writing – original draft, Investigation, Data curation, Conceptualization. **Raj Pavithra:** Investigation, Data curation. **Said Hamid Thangal:** Software, Formal analysis. **Selvam Ganapathy:** Software, Data curation. **Packiaraj Gurusaravanan:** Formal analysis. **Perumal Santhanam:** Writing – review & editing. **Subramanian Radhakrishnan:** Writing – review & editing. **Thirunavukkarasu Muralisankar:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors announce that they have no known challenging financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116406.

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